ARIC Manuscript Proposal # 1097r

1. Full Title: Hereditary variation in expression of apolipoprotein C-I: Role in plasma lipoprotein metabolism

b. Abbreviated Title (Length 26 characters): Effects of a new APOC1 SNP

2. Writing Group:

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. NSS [please confirm with your initials electronically or in writing]

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   Address:

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3. Timeline:
   Upon receipt of the data, we will plan on a maximum of 4 months for analysis and two months for the production of a draft manuscript. This manuscript will be emailed to co-authors for review and comments on or before 6 months after receipt of the data.

4. Rationale:
   In a number of recent papers, we have described a common genetic marker (the Hpa I RFLP, alleles H1 and H2) of the apolipoprotein C-I gene (APOC1) that predicts triglyceride, LDL, apolipoprotein B and apolipoprotein C-I levels in African-Americans and in Caribbean Hispanics. We have now discovered a new common genetic variant of APOC1 that alters the highly conserved core polyadenylation signal (AATAAA) to AATGAA. As such, it is highly likely to be functional. About ½ of H2 carriers also carry the new genetic variant and, in preliminary data, this association completely

   1
explained the observed effect of the H2 allele (via genetic linkage disequilibrium), with only a modest independent effect of the H2 allele (and that in the opposite direction). The new variant is quite common, being present in ~10% of Caribbean Hispanics, and perhaps as much as 27% of African-Americans, and may be a significant contributor to the overall lower triglyceride levels that are observed in African-Americans. The new variant is quite rare in the presence of the H1 allele. No instances of the variant “G“ allele were found in the white Caucasians (European-Americans, N = 162) that we have genotyped to date.

We propose to validate the predictive utility of this new marker with regard to a variety of lipoprotein parameters and also to explore the independent effects of the *Hpa I* RFLP. We anticipate that the clinical/statistical predictive utility of the new marker will be large. Human genetic variation at the population level is only beginning to be defined and reduced to the practical knowledge of interest to physicians. Relevant information on minority populations is particularly scarce. This new genetic variant is common in minority populations and, based on preliminary data, may substantially affect lipoprotein parameters related to the risk of cardiovascular disease.

References:


5. Main Hypothesis/Study Questions:
We will test the hypothesis that a newly discovered common genetic variant of *APOC1* that alters the highly conserved core polyadenylation signal will have important predictive utility with regard to a variety of lipoprotein parameters, including plasma triglyceride and apoB levels. Its association with incident cardiovascular disease may be examined if the number of events among variant strata permits.

Planned Analysis

Sex-specific sample means and standard deviations of un-transformed age, BMI, TG, TC, LDL, HDL, and apoB values will be computed and presented. Subjects will then be cross-classified by *APOE* (ε3/ε3 vs. ε4/*), *APOC1* (H1/H1 vs. H2/*) and SNP (A
vs. G) genotypes. A chi-square test of independence will be computed as an approximate test for linkage disequilibrium of the alleles at these loci.

The primary hypotheses will be that there will be lowered lipoprotein (triglycerides, possibly LDL) and apoB levels associated with the “G” allele of the APOC1 SNP, that these associations will be independent of both the APOC1 H1/H2 context and the APOE genetic context and that the lipoprotein-level associations observed with the APOC1 H1/H2 alleles will be fully accounted for by linkage disequilibrium to SNP A/G. Interestingly, we've not seen any effect on HDL in preliminary data but we will, of course, look for this as well. Lipoprotein/apolipoprotein levels and cardiovascular events (incident MI/Fatal CHD, coronary revascularization, and ischemic stroke) related to the A/G SNP will be compared, individually, in the group as a whole first, then separately in the ε3/ε3 and ε4/* contexts, and then after further stratification by APOC1 H1/H1 and H2/* genotype. The effects of APOC1 H1/H1 and H2/* genotype will be analyzed similarly: first in the group as a whole, then separately in the ε3/ε3 and ε4/* contexts, and finally after stratification by SNP A/G genotype. The small number of ε2/* individuals will be excluded from these analyses. Comparisons also will be made after further stratification by gender, where this variable is found to have a significant effect.

In order to further adjust for other potentially influential covariates, we will fit mixed effects regression models for the outcome variables, with separate terms for each of the combinations of APOC1 SNP A/G, APOC1 Hpa I genotype (H1/H1 and H2/*), and APOE (ε3/ε3 and ε4/*), subject age, sex, BMI, and an indicator variable for recruitment site as fixed effects. Statistical tests will be carried out by comparing the regression coefficient estimates associated with the SNP A/G genotype groups.

6. Data (variables, time window, source, inclusions/exclusions):

- Total plasma cholesterol, LDLc, HDLc, HDL2c, HDL3c, triglycerides, Lp(a), apoA-I and apoB, glucose, waist and hip circumferences, fasting insulin levels, age, sex, height, weight, BMI, race, ARIC community, smoking, and education level data, blood pressure, use of medication for hypertension, diabetes or hyperlipidemia, the diabetes derived variable, physical activity, alcohol use and fibrinogen - all available measures at visits 1, 2, 3, and 4. Multiple measurements will allow us to increase our statistical power by increasing the accuracy of ascertainment and also permit us to examine the effects of genotype on the change in these variables over time. We will also need prevalent and incident MI/Fatal CHD, coronary revascularization and ischemic stroke, to be used as endpoints.

APOE and APOC3 genotypes are needed (so we can compare the relative contribution to predicting TG levels in this population of the well-described APOC3 genotypes and these two new APOC1 genotypes). The effects of the APOC1 genotypes will be impossible to evaluate without the APOE genotype data; these data will be crucial for the project. The APOC3 genotype data will only be useful to us if there is a sufficiency of data on African-Americans. The published report was limited to data from "white men and women" (Surguchov AP, Page GP, Smith L, Patsch W, Boerwinkle E. Polymorphic markers in apolipoprotein C-III gene flanking regions and hypertriglyceridemia. Arterioscler Thromb Vasc Biol. 1996 Aug;16(8):941-947).
We would also like to request access to Dr. Sharrett's post-prandial lipidemia data (Sharrett AR, Heiss G, Chambless LE, Boerwinkle E, Coady SA, Folsom AR, Patsch W. Metabolic and lifestyle determinants of postprandial lipemia differ from those of fasting triglycerides: The Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol. 2001 Feb;21(2):275-281). Altered expression of apoC-I could have a major effect on the extent of post-prandial lipidemia. To evaluate the data we will need access to the carotid intimal-medial thickness – based case/control assignments of the subjects, as described in the article: “Cases had thickness values above the 95th percentile of the ARIC distribution (the criterion relaxed to the 90th percentile for blacks to obtain enough cases for study), and control subjects had thickness values below the 75th percentile in all artery segments evaluated.” We will also need the plasma total cholesterol, TGs, and HDL cholesterol measurements, the apoB-48/apoB-100 ratio in the TG-rich lipoproteins, fibrinogen levels; insulin, glucose levels and the smoking, diet, creatinine, and alcohol data obtained as part of this study. In terms of the actual post-prandial measurements, we will need the “Postprandial responses … calculated as the incremental area under the curve (AUC) defined by TG, RP, or apoB-48 levels at time 0 and at 3.5 and 8 hours after the test meal,” as described in the article. Once again, this specific request is contingent on the presence of a sufficient number of African-American subjects so as to permit meaningful analysis limited to this group.

7.a. Will the data be used for non-CVD analysis in this manuscript?  ___ Yes  ___ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? ___ Yes  ___ No
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?  ___ Yes  ___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”?  ___ Yes  ___ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at:  http://www.cscc.unc.edu/ARIC/search.php

___ Yes  _______ No
10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?
Novel Genes Influencing Cardiovascular Disease Risk in the Population-at-Large: The ARIC Study (Carotid MRI).  2004.11 Boerwinkle, E

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  
Yes  X No
(unless the following is considered an ancillary study: Sharrett AR, Heiss G, Chambless LE, Boerwinkle E, Coady SA, Folsom AR, Patsch W. Metabolic and lifestyle determinants of postprandial lipemia differ from those of fasting triglycerides: The Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol. 2001 Feb;21(2):275-281)

11.b. If yes, is the proposal
  A. primarily the result of an ancillary study (list number* _________)
  B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _________ _________ _________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.